Treatment of Nitrite-Induced Methemoglobinemia with Hyperbaric Oxygen[1] (35846)

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In methemoglobinemia, the tissues are subjected to hypoxia resulting from a decreased pO_2 supplied by the limited amount of oxyhemoglobin. High pressure oxygen (HPO) at 3 atm absolute (ATA) could supply the needed oxygen, physically dissolved, to meet the aerobic demands of most of the tissues (1–3).

Jamieson (4) and Mustala and Azarnoff (5) have shown that oxygen at a pressure of 4 ATA is required before a change in the pharmacological action of some drugs is noted. It seemed worthwhile, therefore, to use oxygen at 4 ATA in evaluating its effectiveness in reducing the lethality and methemoglobinemia produced by a direct hemoglobin oxidant.

Materials and Methods. Female Charles River rats, weighing 175–210 g, were used throughout the study. Sodium nitrite, dissolved in water, was administered to the rats by the intraperitoneal route or added to erythrocyte suspensions at the dose levels indicated in Figs. 1–3. A maximum likelihood probit analysis (6) programmed for computer (7) was used to determine LD_{50} values. After receiving sodium nitrite, the rats or erythrocyte suspensions were subjected to HPO (4 ATA) and maintained at 25 ± 1° for the rats or 37 ± 1° for the cells with an oxygen flow rate of 1 liter/min/rat or 1 liter/min for the cells. Methemoglobin levels were determined in whole blood or erythrocyte suspensions using Storer and Coon's modification (8) of the Evelyn and Malloy technique (9). Erythrocyte suspensions were prepared according to the method of Stolk and Smith (10) with, or without, added glucose at a final concentration of 12 m*M* in Krebs–Ringer phosphate buffer, pH 7.4.

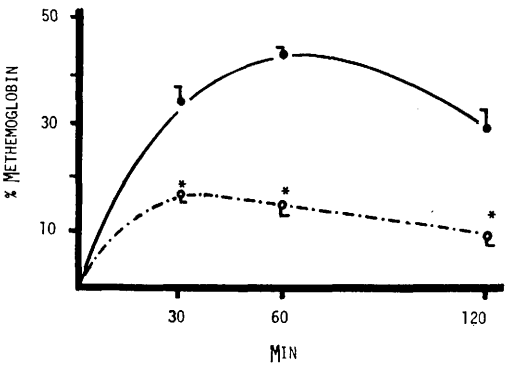

FIG. 1. Effect of HPO on nitrite-induced methemoglobin levels: Rats received sodium nitrite (40 mg/kg ip): controls (●) maintained at normobaric pressure, while treated rats (○) maintained at 4 ATA O_2: $*p<0.01$.

Results. The acute ip LD_{50} value for sodium nitrite was 76 ± 2 mg/kg. After receiving sodium nitrite at a dose of 100 mg/kg ip, rats developed cyanosis within 15 to 30 min. Six out of eight rats died within the first 2 hr and one additional rat died within the next 22 hr. Similarly treated rats exposed to HPO for 2 hr did not appear to be cyanotic and no deaths were observed within a 10-day period following drug administration. The mortality in the rats receiving only sodium nitrite (7/8) was significantly different ($p<0.02$) from that in the rats receiving nitrite plus HPO (0/6).

Figure 1 shows the levels of methemoglobin in rats injected with sodium nitrite (40 mg/kg ip) with, or without, HPO treatment. Rats receiving HPO treatment had significantly lower levels of methemoglobin.

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Since sodium nitrite is a direct hemoglobin oxidant, the action of HPO on nitrite-induced methemoglobinemia must be on the erythrocyte itself. Possible sites of action are: (a) decreased oxidation of hemoglobin; and (b) increased reduction of methemoglobin. *In vitro* experiments in erythrocyte suspensions were conducted to elucidate the site of action. Figure 2 shows the effects of HPO on nitrite-induced oxidation of hemoglobin in erythrocyte suspensions. Methemoglobin levels were measured in the cell suspensions incubated in glucose-free buffer and sodium nitrite at a final concentration of 1 m*M*. Cells incubated under an atmosphere of 4 ATA O_2 had significantly lower levels of methemoglobin than cells incubated at normobaric conditions.

Erythrocyte suspensions were prepared from rats at the time of peak methemoglobin production, 60 min after a dose of sodium nitrite (40 mg/kg ip), and incubated at 37° in buffer containing glucose. Figure 3 shows that there was no significant difference in the rates of methemoglobin reduction between nitrite-induced methemoglobinemic cells exposed to normobaric air or HPO.

Discussion. This work demonstrates that HPO at 4 ATA is effective in reducing sodium nitrite induced mortality and methemoglobinemia. While HPO supplies physically dissolved oxygen to the tissues during hypoxic conditions (1), it also is effective in lowering the levels of methemoglobin. The lowering of methemoglobin levels with HPO treatment appears to be due to hindering of the oxidation of hemoglobin. HPO had no effect in altering the rate of methemoglobin reduction as originally suggested (11). When the original work was tabulated with the results of the present investigation, there was no significant difference in the rate of methemoglobin reduction between erythrocyte suspensions incubated under normobaric pressure and those incubated with HPO.

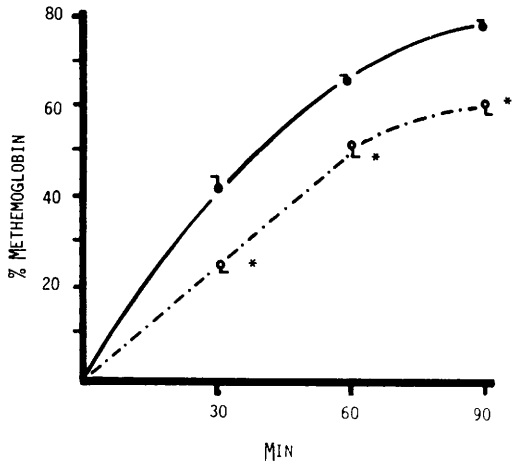

FIG. 2. Methemoglobin levels of erythrocyte suspensions incubated with sodium nitrite at a final concentration of 1 m*M*: controls (●) incubated at normobaric pressure, while treated samples (○) incubated under 4 ATA O_2: $*p<0.001$.

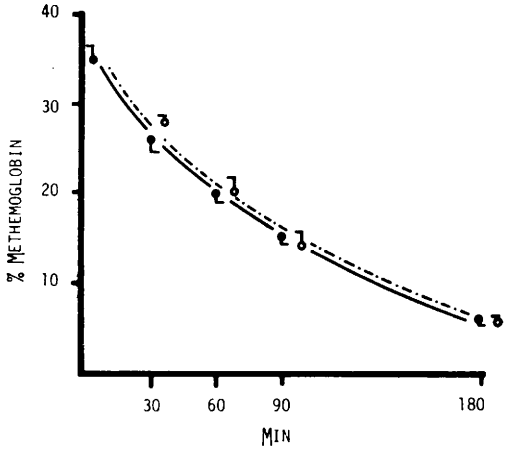

FIG. 3. Methemoglobin levels measured in erythrocytes taken from rats 60 min after an injection of sodium nitrite (40 mg/kg ip): controls (●) incubated at normobaric pressure, while treated samples (○) incubated under 4 ATA O_2.

The mechanism whereby HPO interferes with the oxidation of hemoglobin by sodium nitrite is not known and awaits further investigation. HPO might be acting by producing a conformational change in hemoglobin, making it more resistant to oxidation. It has been suggested that HPO, causing a shift in the hemoglobin–oxyhemoglobin equilibrium toward oxyhemoglobin, produces conditions less favorable to oxidation (12).

Summary. Hyperbaric oxygen decreases nitrite-induced mortality and methemoglobinemia. The action of HPO in reducing methemoglobin levels is brought about by hindering the oxidation of hemoglobin by nitrite.

HPO had no effect on the erythrocyte reductase systems in reducing methemoglobin.

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